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Research Article

FORMULATION AND EVALUATION NASAL INSULIN GEL

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ABSTRACT

The objective of the present study was to formulate insulin gel for intranasal administration and to evaluate it with respect to its *invitro* release studies and hypoglycemic activity in rabbits. The insulin gel was formulated using chitosan as gelling agent. The *invivo* efficacy of insulin gel administered intranasally was assessed by measuring the blood glucose levels at specified time intervals. The use of bioadhesive nasal gel containing insulin not only promoted the prolonged contact between the drug and the absorptive sites in the nasal cavity but also facilitated direct absorption of medicament through the nasal mucosa. Absorption of the drug through the nasal mucosa was high in the first 0.5 to 1.5 hours of the study with a sharp decline in blood glucose and the relative bioavailability of nasal insulin gel was found to be 19.48% of a marketed subcutaneous solution of insulin. This study further demonstrates that administration of insulin intranasally in gel form is a pleasant and painless alternative to injectable insulin.

Keywords: Insulin, Nasal route of administration.

INTRODUCTION

Advances in biotechnology have made available a large number of protein and peptide drug for the treatment of a variety of diseases. These drugs are unsuitable for oral administration because they are significantly degraded in the gastro- intestinal tract or considerably metabolized by first pass effect in the liver. Even the parenteral route is inconvenient for long-term therapy. Of many alternate routes tried, intranasal drug delivery is found much promising for administration of these drugs. Among them Insulin is one of the most promising drugs to treat type I Diabetes Mellitus.

Insulin is a peptide hormone composed of 51 amino acid residues and has a molecular weight of 5808 Da. It is produced in the Islets of Langerhans in the pancreas. It is a hormone that has extensive effects on metabolism and other body functions. It causes cells in the liver, muscle and fat tissue to take up glucose from the blood, storing it as glycogen in the liver and muscle, and stopping the use of fat as an energy source. It is used medically to treat some forms of Diabetes Mellitus. Patients with type1 diabetes mellitus depend on external insulin for their survival because the hormone is no longer produced internally. Some patients with type2 diabetes may eventually require insulin when other medications fail to control blood glucose levels adequately.

Currently, insulin administration requires subcutaneous (sc) injection, which even in the simplest form (Nova-Pen system, Novo Nordisk, Bagsvaerd, Denmark) is cumbersome and unacceptable to many patients with diabetes.

However it is every patient's dream to have access to insulin without the pain of injection. This desire has motivated the search for novel therapeutic approaches to replace the present parenteral insulin delivery. Ideally, an oral insulin dosage form would be preferred over the currently available parenteral route of administration, but this novel approach is confronted by common biological and physicochemical problems such as luminal degradation, particle aggregation, and polypeptide degradation in the absorptive area of the gastrointestinal tract. Several new and alternative routes including nasal, pulmonary, buccal, ocular, rectal, vaginal, transdermal, and others have also been explored for noninvasive delivery of insulin. Out of them nasal route of administration is one of the promising noninvasive routes of administration owing to its high vasculature and other associated advantages like absence of first pass and pH related degradation of Insulin.

MATERIALS AND METHODS

Materials

Human insulin was a gift from Torrent pharmaceuticals ltd, Ahmedabad. Chitosan (low molecular weight) was kindly given by Indian Fisheries department, Kerala. Glucose oxidase kit, Ethylene diamine tetra acetic acid, sodium hydroxide flakes, sodium chloride were purchased from Himedia chemicals ltd. Glycerol, ethanol (absolute), acetic acid and concentrated hydrochloric acid were purchased from sd-fine chemicals ltd.

Equipment

UV- Visible Spectrophotometer (Elico instruments), Centrifuge (Remi instruments), pH meter (Global instruments), Micro pipettes (Finn pipettes, Mumbai), Magnetic stirrer (Remi instruments), Bath sonicator-Model D150H of mrc-Ultrasonic cleaner, Israel.

Preparation of the gel[1]

Insulin was dissolved in an aqueous 2% v/v acetic acid solution. To the clear solution chitosan was added gradually and stirred gently for 20 min and it was kept aside for 1hr to allow the polymer to swell. Then the permeation enhancer ethylene diamine tetra acetic acid (EDTA) was added and stirred gently with a glass rod for 10min. Then the preparation was kept under vacuum to remove the air bubbles if any. In order to get an optimized formulation three formulations were prepared with 2, 3 and 4 % w/v of chitosan.

In vitro drug release studies of the gels[2]

1 ml of the gel was taken into a small test tube. The open end of the test tube was closed with the nasal membrane of the pig by tying it with a thread. Then this was placed in a beaker containing the media i.e. phosphate buffer of pH-6 of volume 25ml such that the membrane was dipped into the media. Then at regular intervals of time i.e. 0.5hr, 1hr, 1.5hr, 2hr, 3hr, 4hr, 5hr, 6hr and 8hr samples of volume 0.5ml were withdrawn using tuberculin syringe and then replaced using the media. The samples then were analyzed using UV Spectrophotometer at the wavelength of 214nm.

In vivo bioavailability studies[3]

The *invivo* studies were conducted in rabbits for which the permission was obtained from the Animal Ethical Committee of Kakatiya University. Rabbits were selected for this study because of the ease of administration of the nasal preparation and to avoid the sacrifice of the animal since this study in rats involves induction of

diabetes and finally sacrifice of the animal whereas the same study in rabbits can be conducted in healthy animals without any induction of diabetes and sacrifice of the animal. Six rabbits were divided into two groups each containing three animals. To one of these groups 100µl of nasal gel containing 4IU of insulin was administered and to the other group 100µl of Human Actrapid solution containing 4IU of insulin was injected. Then the blood samples before the treatment and after the treatment were collected from the marginal ear vein of the rabbit. The serum of these blood samples was separated by centrifugation at 3000rpm for 15min. The serum glucose levels of these samples were estimated by glucose oxidase method using glucose oxidase kit. The colour produced was analyzed using spectrophotometer at 505nm wavelength.

RESULTS AND DISCUSSION

Standard Graph of Insulin in Phosphate Buffer (Ph-6)

Standard graph of insulin was constructed in phosphate buffer (pH-6) by taking the concentrations in the range of 10µg/ml to 70µg/ml and the readings of the absorbance were taken at 214nm in a UV spectrophotometer. The graph was linear with a correlation coefficient of 0.9959 (Fig. 1).

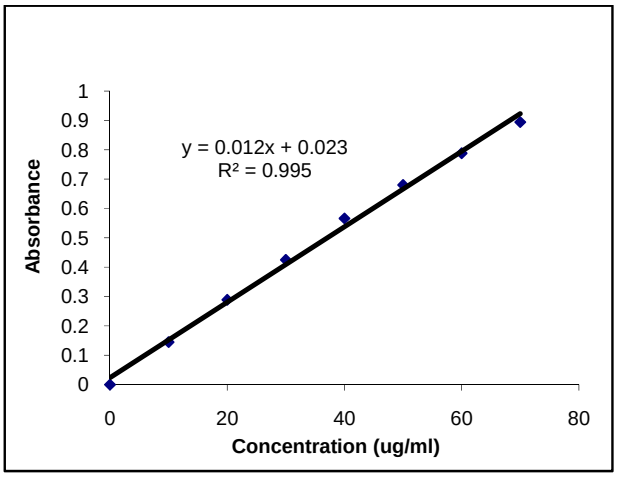

Fig 1: Standard graph of insulin in phosphate buffer(pH-6)

In vitro Permeation studies of Insulin gels

Inorder to eliminate the interference of the proteins of the nasal membrane of the pig and other components of the gel, the release studies were also conducted for their respective blank gels without the drug. From the difference of the absorbance obtained at 214 nm, the amount of insulin released at different time points was calculated using the straight-line equation of the standard graph.

Optimization of the gels was mainly based on viscosity of the formulation and their permeation studies. With 1% of chitosan the prepared gel was found to be very less viscous and there is no three dimensional network. So the optimization was carried out with 2, 3 and 4% chitosan. From the permeation studies 2% chitosan formulations were eliminated, this is due to the rapid release of the entrapped insulin from the gel formulation this may be due to the insufficient viscosity of the gel. Further formulations were attempted with the 3% chitosan, from the in vitro evaluations it was observed that the drug release was observed for a period 6 hours and also the consistency of the gel formulation was found to be optimum for the nasal administration. Later it was also tried with the 4% chitosan, but the release was extended for a period of 8 hours and also the viscosity of the formulations was very high, which may not be suitable for the nasal administration. Release profile of the optimized formulation was aptly fitted into Higuchi model of drug release with a correlation coefficient value of 0.9770. So the further *in vivo* studies were carried out with the 3% chitosan gel formulations (Table 1, Fig. 2).

pH of the gel[4]

3% chitosan gel was formulated at a pH of 3 even though the pH of the nasal fluids was 6 because of following reasons:

a) High absorption of insulin at this pH which can be attributed to its existence as unionized species at this pH.

b) High solubility of chitosan at this pH since it is a cationic polymer and insoluble in neutral and alkaline pH values

Viscosity of the gel

The viscosity of the 3% chitosan nasal insulin gel was determined by Brookefield viscometer, which is a cup and bob type of viscometer with a spindle of size 56 at a torque level of 71% and an rpm of 0.02. The viscosity determined was 13,000 cps.

Table 1: Comparative *in vitro* cumulative % release of insulin from gels of different chitosan concentration

Time (Hr)	Cumulative % release of insulin		
	2% GEL	3% GEL	4% GEL
0	0	0	0
0.5	26.64±1.84	16.87±1.22	8.51±1.12
1	47.57±1.66	30.83±1.43	21.06±1.08
1.5	61.52±1.42	46.10±0.84	33.60±1.42
2	74.07±1.52	57.33±0.68	40.59±0.96
3	90.80±0.82	75.46±1.26	55.94±1.18
4	99.20±0.40	88.01±1.63	65.70±1.64
5		96.39±1.04	74.07±1.28
6		99.17±0.60	82.44±0.98
8			95.00±0.46

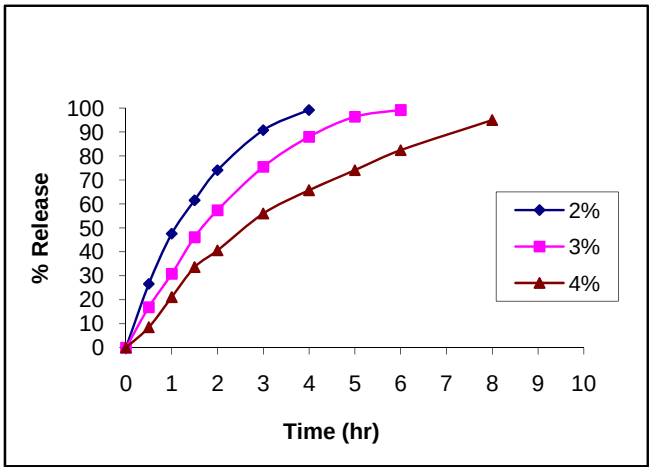

Fig. 2: Cumulative % release of insulin from different chitosan gels

***In vivo* bioavailability study**

A relative bioavailability study between the prepared nasal insulin gel and human actrapid solution containing crystalline zinc insulin was performed. There was a sudden and drastic decline in the serum glucose levels with subcutaneous injection whereas there was a gradual decrease in the serum glucose levels with nasal administration was observed. The time required for the nasal gel to attain the lowest serum glucose levels was around 1.5 hr to 2 hr whereas for the subcutaneous formulation it was 3 hr.

Even though the *invitro* release of the insulin from the gel was 6 hrs, the sustained release was not correlated *invivo* because of the influence of various factors like the rapid passage of the drug into the systemic circulation because of the high blood capillary network of nasal mucosa, mucociliary clearance of nasal cavity which clears the drug and prevents it from entering the systemic circulation, thickness of nasal mucosa and other physiological conditions of the animal.

From the above graph showing the % lowering of blood glucose levels by the nasal insulin gel and human actrapid solution, the areas under the curves were calculated using Trapezoidal rule and from these areas, the relative bioavailability of nasal insulin gel was found to be 19.48% of the human actrapid solution (Table 2, 3 and Fig. 3, 4).

Table 2: Comparative *in vivo* study of nasal gel with subcutaneous marketed product

Time (hr)	Glucose (mg %)	
	Subcutaneous	Nasal
0	77.0±9.2	75.3±8.4
0.5	44.3±6.1	62.1±5.6
1	34.0±5.4	55.2±3.6
1.5	28.4±4.9	47.7±4.2
2	22.0±4.2	59.4±5.6
2.5	17.6±3.8	67.6±6.4
3	30.9±3.4	72.9±4.6
4	49.4±5.2	74.3±6.8
5	73.1±7.1	75.1±6.5

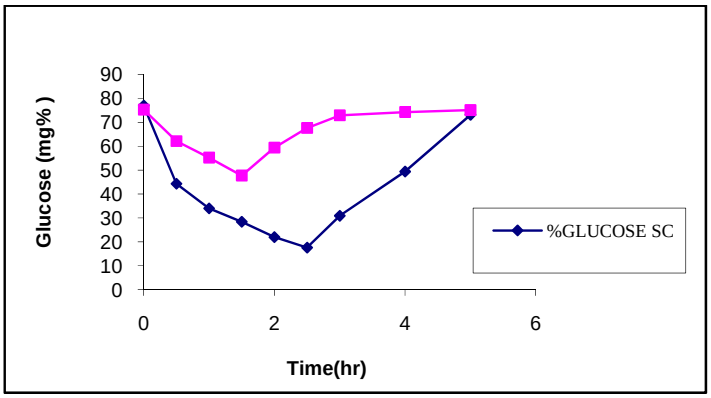

Fig. 3: Comparative *in vivo* study of nasal gel with subcutaneous marketed product

Table 3: Comparative *in vivo* study of % lowering of blood glucose

Time (hr)	% Lowering of glucose	
	Subcutaneous	Nasal
0	0	0
0.5	42.5	17.5
1	55.9	26.7
1.5	63.1	36.6
2	71.5	21.1
2.5	77.8	10.2
3	60.8	3.2
4	35.8	1.4
5	5.1	0

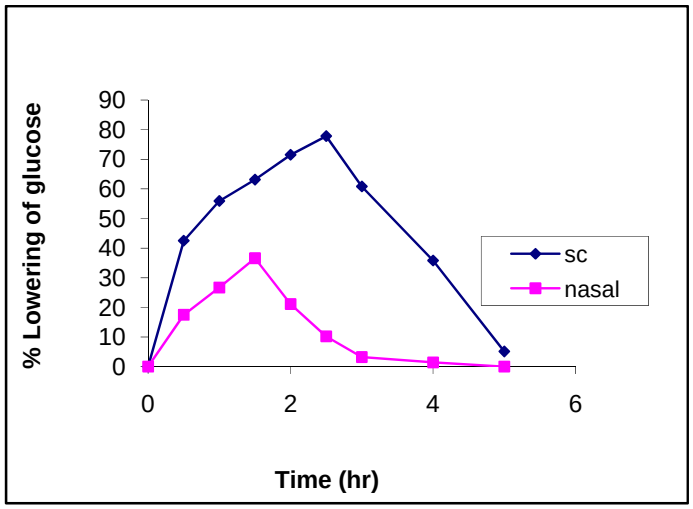

Fig. 4: Comparative *in vivo* study of % lowering of blood glucose

CONCLUSION

This study demonstrates that when insulin is administered in a gel form with a penetration enhancer, it traverses the nasal mucosa and rapidly passes into the systemic circulation. Further, insulin gel delivered via nasal mucosa is a pleasant and painless alternative to injectable insulin. However, as the absorption is quite quick, using this form of insulin delivery may not be feasible for chronic patients in the long run.

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